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Chamber Effect on Growth and N₂ Fixation of *Sesbania aculeata* (L.) At Two Salinity and Moisture Regimes

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Abstract: A greenhouse experiment was conducted to assess the chamber effect on growth and nitrogen fixation in *Sesbania aculeata* (L.) at two levels each of soil salinity (electrical conductivity 4.65 and 7.23 dS m⁻¹) and moisture (15 and 25%, v/w). The plants were grown either in the open or placed in an open-top polyethylene chamber and harvested 6 weeks after seed sowing. Salinization of soil led to a decrease, while higher moisture caused an increase, in different growth attributes of the plants. Growth reduction due to salt stress was less than that due to low moisture stress. Plants kept in the open-top polyethylene chamber showed better growth than those placed outside (in the open); all the parameters studied were better in the chamber. Chamber effect measured as Biomass Enhancement Ratio (BER) averaged 1.22 for different salinity and moisture treatments. However, root biomass of plants grown in the chamber was less than those grown in the open. Negative effect of salinity and low moisture was mitigated to a significant extent under chamber conditions. The analysis of root and shoot material for total N and ¹⁵N showed significant amounts of N₂ being fixed as measured by isotopic dilution technique. Significantly higher amounts of N were fixed at 25 than 15% soil moisture; soil salinity had a depressing effect on the amount of N₂ fixed. The contribution of biologically-fixed N (Ndfa) to the total N content of shoot and root portions was 23-62% and 21-52%, respectively, under different growth conditions. Contribution of Ndfa decreased with salinity but was more at 25% than 15% soil moisture.

Key words: N₂ fixation - rhizodeposition - rhizorespiration - elevated CO₂ - ¹⁵N, - isotopic dilution

INTRODUCTION

Open top chambers have frequently been used to study the effect of elevated carbon dioxide (CO₂) on plant growth, rhizodeposition and rhizospheric microflora and microbial functions. In these studies, enclosure of plants in the chambers is reported to have a significant positive effect (chamber effect) on growth and other attributes of ecosystem functioning (Sgheri *et al.*, 1998; Mayr *et al.*, 1999). Reduction in wind speed and turbulence relative to outside are considered to be the most important contributors to the chamber effect (Owensby *et al.*, 1993). The resulting increase in humidity of the chamber will also lead to a reduction in evapo-transpiration and improved water use efficiency vis-à-vis stomatal responses. In addition, CO₂ content of the chamber atmosphere relative to outside will certainly increase as a result of rhizorespiration (microbial and root respiration) and efflux of the resulting CO₂. A substantial efflux of CO₂ from soil is a common feature (Azam *et al.*, 1985; Baggs *et al.*, 2003; Pumpanen *et al.*, 2003). This efflux will be sufficient to raise the CO₂ concentration by several folds within the crop stand. The model proposed by Vaughan and Suarez (2002) shows CO₂ efflux of ca 100 mg m⁻² hr⁻¹ from a wheat canopy. This efflux will be sufficient to raise the canopy CO₂ level significantly with a substantial beneficial effect on plant performance (Drake and Leadley,

1991; Poorter, 1993; Idso and Idso, 1994; Azam and Farooq, 2001). The effect of CO₂ has been more positive when environmental factors are affecting the plant growth and development severely (Koch and Monney, 1996).

Amongst different plant types, legumes occupy an important position with respect to the functioning of natural and artificial ecosystems through N acquisition from the atmosphere (Zanetti *et al.*, 1997; Hopkins, 1999). In agroecosystems, green manuring legumes occupy a key position in maintaining/improving soil fertility and productivity. Their significance has increased further as a source of supplemental nitrogen due to economic and pollution potential concerns of chemical N fertilizers. Of the different legumes, species of *Sesbania* have generally been considered as most important for green manuring especially in wheat-rice rotation system. In arid regions, this system is characterized with varying degrees of salinity and water stress. However, there are hardly any studies to demonstrate chamber effect vis-à-vis elevated CO₂ on green manuring legumes. Therefore, our objectives were i) to monitor changes in humidity, temperature, and CO₂ outside and inside the chamber and ii) to study the response of *Sesbania aculeata* to different salinity and moisture regimes when grown under normal and changed (with respect to humidity, temperature and CO₂) greenhouse conditions.

MATERIALS AND METHODS

Soil used in the experiment was collected from the top 0-15 cm of experimental plots with a history of receiving ¹⁵N-labelled fertilizer and cultivated to *Sesbania*. As such the soil was not only enriched with ¹⁵N (atom % ¹⁵N of the potentially mineralizable N being 0.3801 as determined by anaerobic incubation method (Stanford and Smith, 1972) but had sufficient number of rhizobia specific to *Sesbania* as suggested by dense nodulation in different experiments conducted previously. Air-dried and sieved (<2 mm) soil had the following characteristics: organic C, 0.6%; total N, 0.055%; NH₄-N, 7 µg g⁻¹ soil; NO₃+NO₂-N, 12 µg g⁻¹ soil; pH (1:1, soil: water suspension), 7.7; electrical conductivity, 4.65 dS m⁻¹; sand, 39.6%; silt, 26.2%; and clay, 34.2%.

Five-Kilogram portions of the air-dried and sieved soil were filled in 6-kg plastic pots and sown to *Sesbania* (5 seeds pot⁻¹ on April 16, 2002); the seedlings were thinned to 3 within 2 days after seed germination. The soil was irrigated with water or water containing NaCl to achieve the variation in soil moisture content of 15 or 25% (v/w) and raise the salinity level to an electrical conductivity of 7.23 dS m⁻¹. Nine pots were used for each treatment with triplicate being placed randomly in the I) greenhouse under ambient conditions and ii) open-top polyethylene chamber, and iii) open-top chamber to serve as control for irrigating the pots placed in (ii). The chamber consisted of an iron-bar frame (1 x 1 x 1.5 m) covered with polyethylene sheet and having an opening on the top to allow for gaseous exchange. Soil moisture content was maintained at required level by making up the weight loss determined in pots placed in one of the chambers used as control for this purpose. Loss of water was always less from enclosed than open pots. Relative humidity, temperature, and CO₂ concentration inside and outside the chamber was monitored twice a day and 8 times during the experimental period of 6 weeks. Air samples obtained using 50-mL air-tight syringe were analyzed for CO₂ on a gas chromatograph equipped with flame ionization and electron capture detectors.

Six weeks after seed sowing, data on number of leaves, maximum leaf length, and plant height were recorded. Uniform leaf samples were taken for the determination of Relative Water Content (RWC) of leaves by using the expression:

$$RWC = (fresh\ weight - dry\ weight) / (turgid\ weight - dry\ weight) * 100$$

Turgid weight was determined by overnight incubation of leaf segments in water under dark conditions and blotting the extra water on leaf samples prior to weighing. The whole plants with intact roots were recovered from the pots by washing away the soil. All the nodules were carefully detached from the roots and

weighed after drying at 65°C. Fresh and dry weight (oven drying a 65°C) of the root and shoot portions was recorded and the plant material finely powdered. Aliquots of the root and shoot material were analyzed for total N using micro-Kjeldahl method (Bremner, 1996). The distillates were acidified with 1M H₂SO₄ (0.1 µL µg⁻¹ N), concentrated to 1 mL at 90°C on a hot plate, and appropriate volumes subjected to N-isotope analyses on a mass spectrometer using KOBBr as alkalizer. Calculations for plant N derived from fixation were made on the basis of ¹⁵N dilution resulting from N₂ fixation. Soil used in the present study was fairly enriched with ¹⁵N (atom % ¹⁵N content of potentially mineralizable N being 0.3801) thereby providing a reliable means of calculating the proportion of plant N derived from soil using the expression:

$$\% N\ derived\ from\ soil\ (\% Ndfs) = \frac{Atom\ \%^{15}N\ excess\ of\ plant\ N}{Atom\ \%^{15}N\ excess\ of\ soil\ N} \times 100$$

$$\% N\ derived\ from\ fixation = 100 - \% Ndfs$$

Amounts of plant N derived from soil and fixation were calculated by using the values obtained from above equations.

Statistical analyses of the data included determination of standard deviation of the means and coefficient of correlations using MS EXCEL computer software programme.

RESULTS

Temperature, relative humidity and CO₂ were consistently higher in the chamber atmosphere as compared to that outside (Fig. 1). Observations recorded in the morning showed lower values for temperature and humidity but higher CO₂ concentrations. In the present study, higher level of soil salinity had generally a depressing effect on the growth and biomass production of *Sesbania* (Fig. 2). All the growth parameters studied i.e., plant height, number of leaves, longest leaf, biomass of root and shoot, leaf water content, and nodule weight decreased due to salinity under all growth conditions. Relative water content (RWC) of leaves varied between 71 and 85% in different treatments. Lower values were obtained for plants grown in saline soil at lower (15%) moisture content. Under these conditions, a significant positive chamber effect was noted and RWC increased from 75 to 84% and from 71 to 85% at the two salinity levels. The differences were observed mainly at 15% moisture, while at 25% moisture, RWC was almost similar in different treatments. The results presented here suggest that salinity combined with low moisture exerted higher stress; while high moisture fairly mitigated the negative effects of salinity and there was no significant chamber effect either.

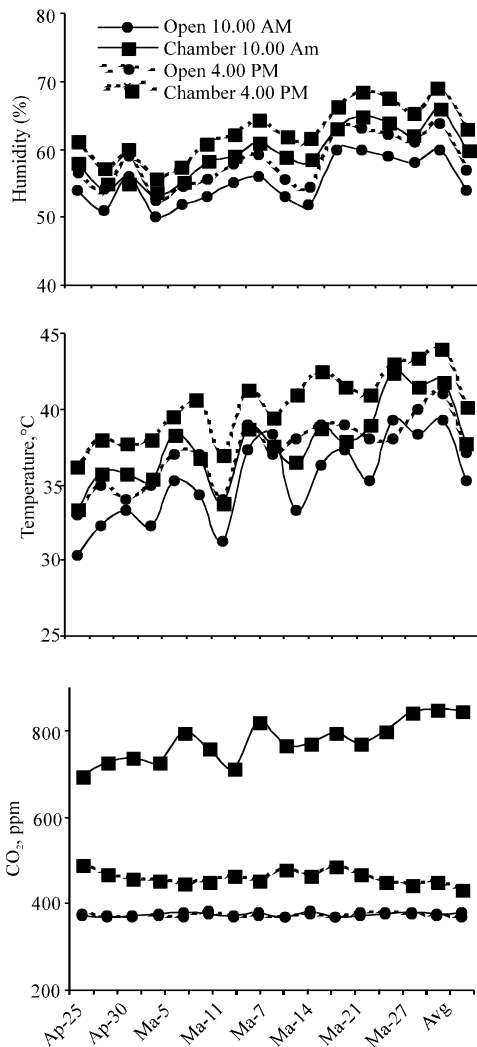


Fig. 1: Changes in humidity, temperature and CO₂ concentration of the atmosphere in and outside the chamber during the months of April (Ap) and May (Ma). Average (Avg) of all the measurements are also plotted

Salinity-induced reduction in growth was more pronounced at lower than that at higher soil moisture content. Growth reduction due to stress (GRS) (Poorter and Perez-Soba, 2001) was found to be 8.6%. Enclosure of plants in the open-top chamber had in general a positive effect (chamber effect) on different plant growth parameters especially under saline and low moisture conditions. Chamber effect expressed in terms of biomass enhancement ratio (BER) (Poorter and Perez-Soba, 2001) i.e., Biomass in the chamber/biomass in the open was 1.22 showing 22% gain in the biomass of plants grown under different conditions of salinity and moisture. Biomass of plants was significantly more at higher than that at lower

soil moisture level (8.5 and 7.1 g pot⁻¹, respectively, when expressed as an average of different treatments) with a negligible chamber effect in the former case; BER for higher and lower moisture regimes being 1.1 and 1.0, respectively. However, when lower moisture was taken as stress factor, GRS was 19%. Of the different plant parameters studied, nodulation as measured by biomass, showed a higher negative (to salinity) or positive (to moisture and enclosure in chamber) response. Chamber effect on nodulation in terms of BER was found to be 28%. Allocation of dry matter and N to roots was higher in plants grown in the chamber than those grown in the open i.e., 35 and 22% compared to 29 and 19%, respectively.

Trends in N content of root and shoot were similar to those observed for dry matter yield (Fig. 3). Computation of data in Fig. 3 and 4 showed a significant positive correlation of 0.77, 0.99, and 0.94 between dry matter and N content of shoot, root and shoot + root, respectively (*n* = 8 in each case). In the present study, roots were found to bear abundant pinkish nodules suggesting the presence of effective rhizobia in sufficient numbers. Hence, it was possible to determine the contribution of biological N₂ fixation to the total N in plants using ¹⁵N isotopic dilution technique with some modification. In practice, a non-leguminous reference crop is required to estimate N₂ fixation in the legume. In the present study, however, the difference in ¹⁵N abundance of soil N and atmospheric N was sufficiently high to enable the determination of percent N derived from soil as described in M & M section. The amount of N fixed varied between 36 and 147 mg pot⁻¹ in different treatments (Fig. 4). Soil salinity had a significant depressing effect with the amount of N fixed being 79 mg pot⁻¹ compared to 102 mg pot⁻¹ under non-saline conditions. Higher moisture had a positive effect on the amount of N₂ fixed with 122 and 59 mg N pot⁻¹ being derived from fixation at 25% and 15% moisture, respectively. A greater proportion of fixed N was allocated to the shoot portion i.e., 77-84% of the total in different treatments. A positive chamber effect (averaging 41%) on the amount of N₂ fixed under different conditions of salinity and moisture was observed.

Contribution of biologically fixed N to the total N content of shoot and root portions (%Ndfa) was 23-63% and 21-49%, respectively, under different growth conditions (Fig. 4). Lowest values were recorded for plants grown in the open at 15% soil moisture and highest values for those grown in the chamber at 25% moisture. On an average, the contribution of biologically fixed N remained low in non-saline soil. Overall contribution of fixed N to the total plant N ranged between 20-60%. A positive chamber effect of 30% was observed for the percent contribution of biologically fixed N to the total N of plants grown under two salinity and moisture regimes.

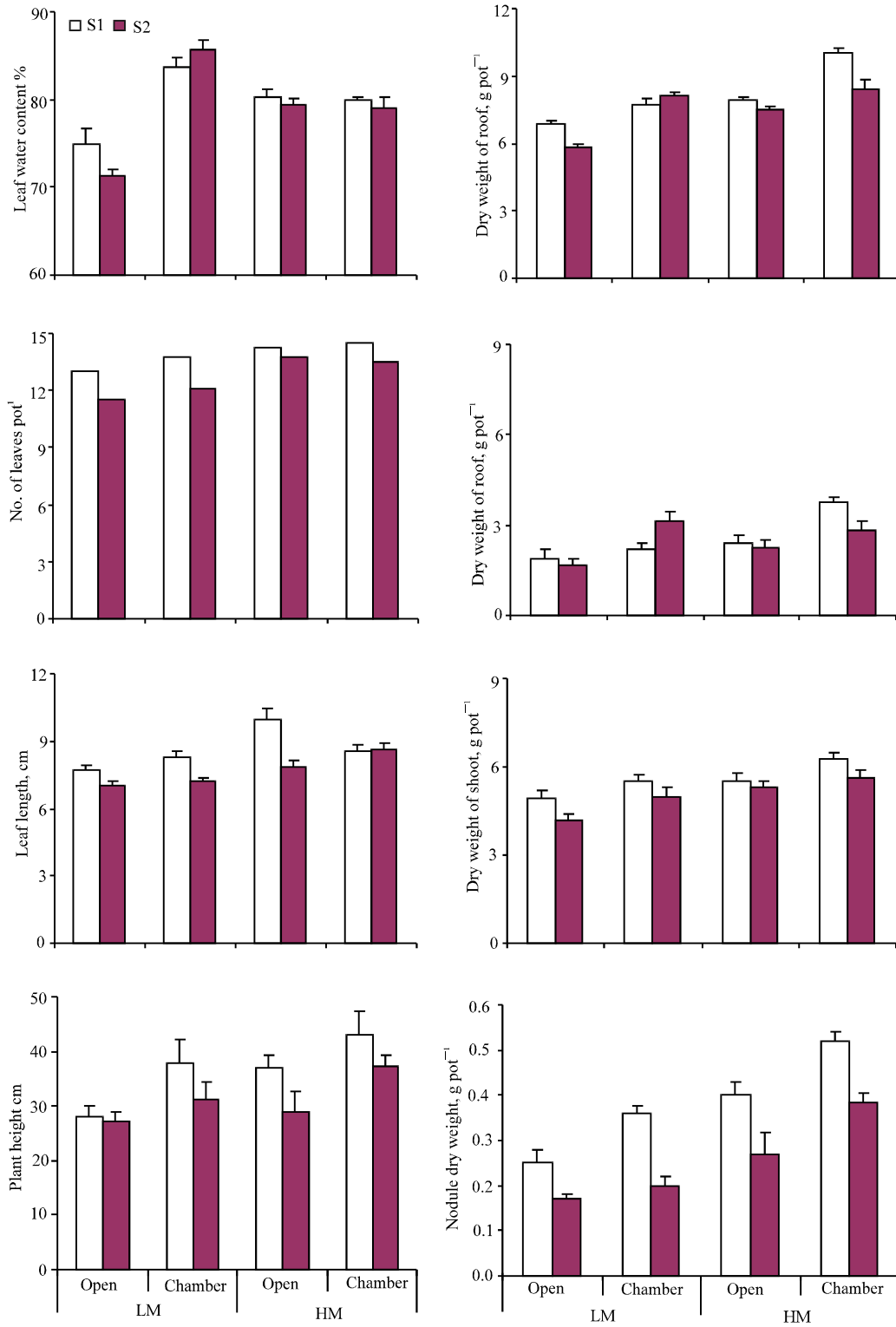


Fig. 2: Effect of salinity (S1, normal; S2, saline) and moisture (LM, 15%; HM, 25%) on different parameters of plants grown outside (open) and inside the chamber; standard error bars are also shown

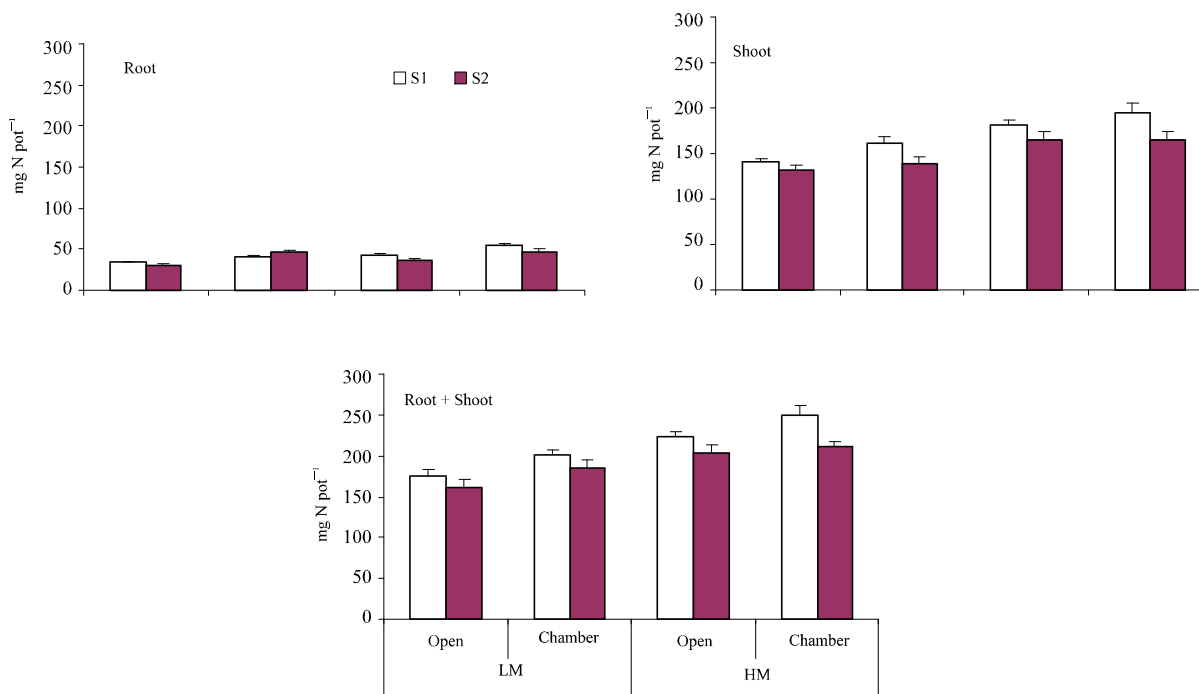


Fig. 3: Distribution of N in root and shoot portions of plants grown outside (open) and inside the chamber under different conditions of salinity (S1, normal; S2, saline) and moisture (LM, 15%; HM, 25%); standard error bars are also shown

DISCUSSION

Atmospheric concentration of CO₂ has increased significantly over the past few decades and is likely to double in the present century (King *et al.*, 1992). The increase could benefit the plants in terms of increased photosynthesis and productivity (Poorter, 1993; Idso and Idso, 1994). The beneficial effects are generally more when plants are facing rhizospheric or atmospheric stresses (Koch and Monney, 1996; Polley *et al.*, 1996). In most of such studies, the plants have been subjected to ambient or twice the ambient concentrations of CO₂ using open-top chambers or free-air CO₂ enrichment facilities. Results presented in Fig. 1 show that plants grown in open top chamber are in fact exposed to elevated levels of CO₂, humidity and, temperature. Increase in CO₂ concentration in the chamber can be attributed mainly to rhizorespiration (includes microbial and root respiration). In most studies, however, contribution of rhizorespiration to CO₂ concentration within the plant canopies/stand has not been given due concentration. This happened in spite of the fact that, efflux of CO₂ from soil is substantial and may potentially raise its level several times the ambient (Baggs *et al.*, 2003; Vaughan and Surez, 2002).

Results presented in this study, show that mere enclosure of plants in an open-top chamber leads to

improved growth and other plant functions, particularly the process of N₂ fixation. Significant chamber effects have been reported by others (Sgheri *et al.*, 1998; Mayr *et al.*, 1999). In addition, stress imposed by salinity and low soil moisture content is mitigated to a significant extent by the conditions created in the chamber, especially a rise in relative humidity and CO₂ concentration (Fig. 1). In the chamber, absence of free flow of wind (Owensby *et al.*, 1993) could favour build-up of humidity leading to changes in evapotranspiration as well as photosynthesis vis-à-vis increase in the levels of CO₂. Maintenance of higher soil moisture level in the enclosed pots was evident from less watering requirement (data not presented) and a higher RWC of leaves (Fig. 2). Besides high humidity, elevated CO₂ is reported to decrease stomatal conductance by 30-60% (Morison, 1993) thus reducing water loss by plants. Therefore, elevated CO₂ in the chamber atmosphere would have alleviated water stress leading to a substantial increase in N₂ fixation and other growth attributes of *Sesbania*. Alfalfa (*Medicago sativa*) plants grown at elevated CO₂ showed a lower decrease in leaf water potential than those grown at normal CO₂ when subjected to water stress; chamber effect was noted (Sgheri *et al.*, 1998). Similar results have been reported by others for different plant species (Rabha and Uprety, 1998; Seneweera *et al.*, 2001).

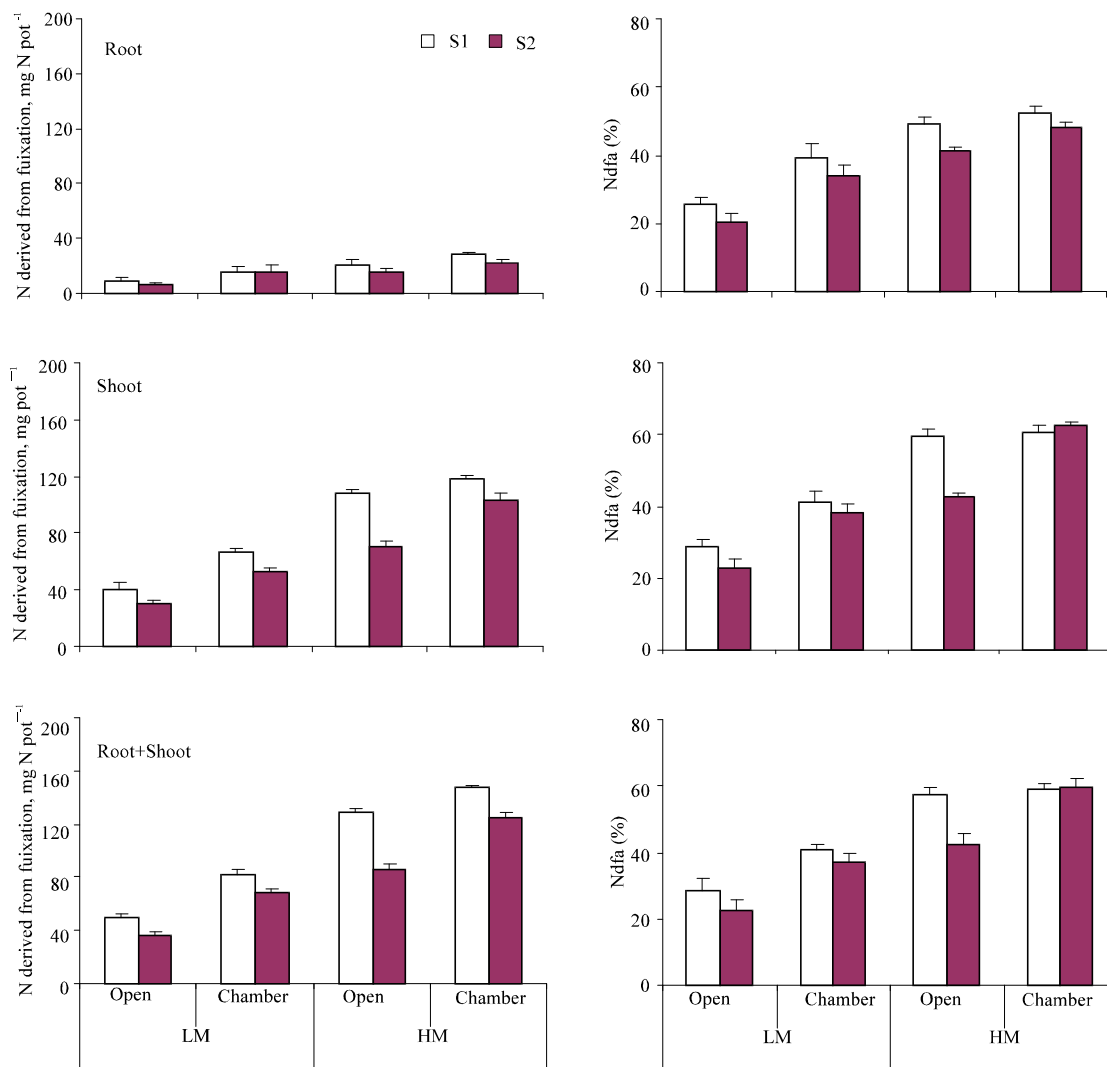


Fig. 4: Contribution of biologically fixed N to the total N of root, shoot and root+shoot of plants grown outside (open) and inside the chamber under different conditions of salinity (S1, normal; S2, saline) and moisture (LM, 15%; HM, 25%); standard error bars are also shown

In the present study, elevated CO₂ would have mitigated the negative effects of increased temperature and humidity in the chamber. Higher moisture level in the root-zone had a positive effect on different growth parameters as well as N content of plants (Fig. 2 and 3). This could partially be attributed to enhanced N₂ fixation under high moisture conditions that limit the availability of oxygen at the nitrogenase level; a condition suitable for efficient N₂ fixation (Sangakkara *et al.*, 1996). Since legumes depend mainly on biological N₂ fixation besides N from soil organic matter, the factors that support the former process will have a positive impact on plant growth. Higher moisture level also mitigated the negative effect of salinity probably because of dilution of salts in

the soil solution. This would also result in better plant growth especially at higher salinity level.

The observed increase in plant growth under chamber conditions could be attributed mainly to increased nodulation and enhanced N₂ fixation (Fig. 4) at the expense of increased photosynthesis under elevated CO₂ conditions. Stimulation of symbiotic N₂ fixation at higher levels of CO₂ (Yu *et al.*, 2002) and a significant increase in different yield parameters of soybean have also been reported (Amthor *et al.*, 1994). Partitioning of photosynthates to the roots, nodule mass, and rhizobial population are also reported to increase at elevated CO₂ levels (Tschaplinski *et al.*, 1993; Drake *et al.*, 1997; Murillay *et al.*, 1999).

Data presented in Fig. 4 shows 53-77% of the plant N being derived through biological nitrogen fixation and the rest (53-77%) from soil. This percentage is relatively lower than that commonly reported, i.e., 80-90% (Eaglesham *et al.*, 1977) but possible as the plants were harvested at an early stage of growth. A higher contribution of biologically fixed N to the total plant N at elevated levels of CO₂ is often attributed to increased C availability for nodule formation and for the functioning of nitrogenase (Tissue *et al.*, 1997; Serraj *et al.*, 1998). An increase in total N yield and the percentage of plant N derived from symbiotic N₂ fixation in *Trifolium repens* under elevated CO₂ (Zanetti *et al.*, 1997).

Different factors had important bearing on the process of N₂ fixation. Soil salinity had a negative effect while high moisture a substantially positive effect on the contribution of biological N₂ fixation to the total plant N. This was attributable to the overall effect of these factors on plant growth. Reduced plant growth with increased salinity would lead to less photosynthesis and partitioning of photosynthates to the soil to support N₂ fixation at higher salinity level. Enhanced rates of photosynthesis, on the other hand, should allow greater partitioning of carbohydrates below-ground, thereby enhancing root growth and enabling plants to better explore the soil for water and nutrients (Wall, 2001). In the present study, high moisture promoted the contribution of N₂ fixation to N nutrition of plants. Contribution of BNF (biological nitrogen fixation) decreased with salinity, but elevated CO₂ arrested the decrease to a significant extent. Such benefits are reported to be derived from the availability of more solutes for osmoregulation and from reduction in the transpirational intake of salts (Bazzaz, 1990). Munns *et al.* (1999) observed Salinity and CO₂ interaction at low but not at high salinity have been reported (Munns *et al.*, 1999). In addition, positive effect of elevated CO₂ on root proliferation and root-induced microbiological and biochemical changes may help plants withstand salinity stress.

Results of this study suggest a positive effect of elevated CO₂ on growth and N₂ fixation of *S. aculeata* grown under mild salinity and high moisture. It is possible, therefore, to enhance the biomass yield of this green manuring crop by elevating the level of CO₂ in the plant canopy. Under practical agricultural conditions, plant residues decomposing on the soil surface following mulching may help elevate the level of CO₂ and thus the plant growth.

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